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FS-SO-1102-1.130
(Problem 1)

FINAL REPORT

ADMINISTRATIVE STUDY OF ENDRIN HAZARD TO WILDLIFE WHEN DIRECT SEEDING

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Final Report Summary
W. L. Thomas - Atlanta, Ga., and
W. F. Mann, Jr. - Alexandria, La.

ADMINISTRATIVE STUDY OF ENDRIN HAZARD TO WILDLIFE WHEN DIRECT SEEDING

Four test areas ranging from about 75 to 100 acres were sown with repellent-treated seed and censused for dead birds and mammals four or five times. Endrin levels in birds and rodents were determined in specimens taken before sowing and after the last survey, as well as from any dead specimens found. It was a cooperative study with the U. S. Fish and Wildlife Service; in Louisiana, the State Wildlife and Fisheries Commission also participated. All tests were conducted on National Forest lands in Florida and Louisiana, with Ranger District personnel assisting.

Results were not conclusive or consistent. In general, highlights might be summarized as follows:

1. Some bird and mammal specimens contained endrin before sowing. The percentage of specimens taken about 30 days after sowing containing endrin was higher than before sowing on three of the four test sites.

2. On two areas, no kill of birds or mammals was detected.

3. On the other two areas, kills were 2 birds and 2 mice on one, and 7 birds on the other.

4. All areas except one had high bird and mouse populations, so kills were minimal in relation to probable exposure.

Recommendations are given to minimize hazards to wildlife when direct seeding.

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INTRODUCTION

In many parts of the country seed-eating forest rodents, birds, and insects are a major deterrent to direct-seeding efforts. Landowners commonly coat seeds with Arasan, endrin, and aluminum powder, using a latex sticker, for protection from these predators. Arasan or thiram (tetramethylthiuram disulfide) serves as a bird repellent, endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5-8-dimethanonaphthalene) is a rodent repellent and insecticide, and the aluminum overcoating is included to lubricate the seed. Endrin is the

most toxic of the chlorinated hydrocarbons. LD-50 (95 percent confidence limit) for formulations with 95 and 96 percent technical purity ranges from 0.75 for grouse to 17.8 for male white rats 1/, 2/.

The U. S. Forest Service stopped using endrin in the repellent formulation for direct seeding when Executive Order No. 11643 of February 1972, "Environmental Safeguards on Activities for Animal Damage Control on Federal Lands," was issued because it was feared that primary, and perhaps secondary, hazards to wildlife existed. Sowing operations in the southern National Forests were continued using only Arasan, a bird repellent, for seed protection. After endrin usage ceased, failures became more prevalent due to seed loss to depredating rodents and insects. Corporate owners, on the other hand, have not curtailed the use of endrin since the Executive Order applied only to Federal programs. Consequently, endrin is still used by private owners at its registered 0.5 pound (technical grade) per hundred pounds of seed for direct sowing. This is half the dosage used prior to 1971, when it was found that endrin in the repellent formulation could be reduced substantially.

To estimate hazards to wildlife as a basis for formulating a specific policy, the U. S. Forest Service initiated a study in the South in the winters of 1973 and 1974. It was a cooperative study between the U. S. Fish and Wildlife Service (FWS), National Forest Service (NFS), and the Southern Forest Experiment Station (SFES). Longleaf and loblolly pine were sown on four experimental tracts to determine if repellent-treated seeds endangered birds and mammals.

1/ 1970. Richard K. Tucker and Crabtree, D. G. Handbook of toxicity of pesticides to wildlife. USDI, Denver Wildlife Center Resource Publication No. 84.

2/ 1959. S. H. Kerr and Brogden. Relative toxicity to mammals of 40 pesticides. Agricultural Chemicals, Vol. 14, No. 9.

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PROCEDURES

Longleaf pine and loblolly pine sites were studied in Florida and Louisiana according to the following schedule:

Longleaf

Florida	November 1973
Louisiana	November 1973
Louisiana	November 1974

Loblolly

Louisiana	January 1974

All study areas were located on National Forest lands and were approximately 75 to 100 acres each. Sites were prepared in accordance with standard prescriptions and seeded by aerial sowing except for one of the Louisiana longleaf sites which was sown with the Hatcher Seeder that normally covers a major portion of the seed with soil. Sites selected were relatively free of deep sands and wet flats.

Seeds were coated with Arasan-endrin-aluminum flakes and a latex sticker as prescribed in USDA Handbook No. 391. Stauffer's Endrin 50-W was used in the formulation at the registered rate of 0.5 pound of technical grade endrin per 100 pounds of seed. Longleaf seed was sown at the rate of 3 pounds per acre, based on uncoated weight, on broadcast areas and about 1½ pounds per acre on the row-seeded area. Loblolly seed was sown at the rate of 1 pound per acre, based on unstratified and uncoated weight.

Pretreatment bird and mammal collections were conducted on areas near test sites where topography, soil type and vegetational cover were similar to the test plots. Bird species were collected according to their availability with an emphasis on those most prevalent on the study area. Attempts were made to collect five birds of each species present. At least 100 traps were set each night for several nights prior to each seeding to collect small mammals.

Transects ½ chain (33 feet) apart, running the width of the test fields, were marked prior to sowing. Every fifth transect was searched for sick or dead birds and mammals with the first strip randomly selected from the first five, ½-chain strips. Surveys of the selected transects were conducted by 4-man teams walking the length of the transects. The search was extended 50 feet into the adjacent border at each end of the searched transects.

At each test area, attempts were made after the last post-treatment survey to collect five birds of each species or species group which were common to the area before or after seeding. In no case was this achieved.

To collect small mammal specimens, 100 snap traps baited with peanut butter and rolled oats were set at each test area the day before the last survey.

Fish samples were collected at the one site which had a stream in the seeded area. Searches were made for dead or dying aquatic organisms during each of the surveys.

All specimens were tagged to show collection information, wrapped in aluminum foil, put into plastic bags and quick frozen on dry ice. Later storage was in a deep freeze until shipped on dry ice for laboratory analysis.

Samples were analyzed for endrin and delta-keto endrin at the USDA Environmental Monitoring Laboratory in Gulfport, Mississippi.[1/] When more specimens of a given species were collected than were needed for residue studies, the desired number were drawn at random. All dead specimens found on the test sites were analyzed for endrin after careful examination for signs of gunshot wounds, attack by predators or any other nonpesticide related factors which could have contributed to their death.

1/ Details of laboratory procedures are given in a publication and a mimeographed set of instructions that are in the Appendix (Exhibits 1 and 2).

RESULTS

Longleaf Pine Sown in Florida

Site description.--The study was located on the Wakulla Ranger District, Apalachicola National Forest in Florida. More specifically, it was situated in Section 15, R.3 W., T.2 S. The site, selected by the Forest Service, is approximately 97 acres. The soil is Leon fine sand, located in the flat coastal lowlands and is characterized by pine-palmetto flatwoods.

The seedbed was prepared for longleaf seeding by burning and strip disking approximately 1 month prior to seeding. It was judged to be excellent by local forestry personnel.

The area is irregular in shape with the width ranging from 0.10 to 0.40 mile wide and about 0.87 mile long. It is bordered on one side by a Forest Service sand road and the remaining portion is surrounded by longleaf pine and turkey oak. Mast production was abundant in the adjacent areas, providing an ample food supply for wildlife. It is particularly noteworthy that longleaf pine seeds were unusually abundant during the test period.

Biologic surveys.--On November 7, 8, and 9, pretreatment bird surveys were conducted and survey lines were staked out on the test site. Bird species observed in the area are listed in table 1. Bird collections off the site were conducted at various points outside a 2-mile radius of the test fields. The traps remained unset from November 8 to November 12 to reduce trap shyness. The traps were baited and set daily from November 12 to November 18, and totaled 600 trap-nights.

Table 1.--Pretreatment bird observations and collections of birds and mammals

Pretreatment Observations, 11/7-9/73

Pinewood sparrow (Aimophila aestivalis)(Most abundant of birds observed)
White-throated sparrow (Zonotrichia albicollis)
Vesper sparrow (Pooecetes gramineus)
Catbird (Dumetella carolinensis)
Robin (Turdus migratorius)
Red-bellied woodpecker (Centurus carolinus)(in adjacent timber)

Pretreatment Collections

1 Red-bellied woodpecker
1 Catbird
4 Eastern phoebe (Sayornis phoebe)
1 Brown-headed nuthatch (Sitta pusilla)
1 Robin
1 Unidentified sparrow
2 Pinewood sparrow
1 Vesper sparrow
7 Pine warblers (Dendroica pinus)
1 Eastern meadowlark (Sturnella magna)
1 Grasshopper sparrow (Ammodramus savannarum)
2 Eastern bluebird (Sialia sialis)
8 Shorttail shrew (Blarina brevicauda)
2 Hispid cotton rat (Sigmodon hispidus)

Bird collecting was continued on November 12, 13, and on the morning of the 14th. Pretreatment collections are listed in table 1.

Quail censusing was conducted on the study area on November 12 and 13. No quail were seen or heard during this period or in any subsequent post-treatment checks.

After sowing, marked cowbird carcasses were randomly placed in the field test, 15 in the brush along the field edge and 35 in the center, to obtain a measure of scavenging and also a measure of searching success. Known locations were checked to see if "losses" were due to scavengers. Results were as follows:

11/14/74	15 Cowbirds randomly placed in brush along field edge	
	35 Cowbirds randomly placed in center of field	

Birds recovered in 2 days of survey

11/15	4 out of 15	from edge
	14 out of 35	from center
11/16	6 out of 11	from edge
	2 out of 21	from center
Total Found	10 out of 15	from edge
	16 out of 35	from center

Percent either removed by scavengers or not detected in the search:

33 1/3	from edge
54	from center

Aerial seeding was conducted on November 14. Surveys for dead or dying animals were conducted the first, second, fifth, and nineteenth days after sowing. Results of these surveys are listed in table 2.

Table 2.--Post-sowing collection of birds and mammals

Dead in Test Field	
11/16/73	1 Cotton mouse (Peromyscus gossypinus)
11/19/73	1 Bluebird
11/19/73	1 Cotton mouse
12/3/73	1 Eastern meadowlark
Post-sowing Collection	
12/4/73	1 Shorttail shrew
	1 Vesper sparrow
12/5/73	1 Cotton mouse (adult)
	1 Vesper sparrow
12/6/73	2 Cotton mouse
	2 Mourning dove (Zenaidure macroura)
12/7/73	2 Cotton mouse
	1 Robin
12/8/73	1 Cotton mouse
	1 Florida mouse (Peromyscus floridanus)
	3 Robin

Post-treatment collections of birds and mammals from the test field were made between December 3 and 9. These specimens are also listed in table 2.

Results of endrin analyses of specimens collected before and after sowing, and those found dead on the study area are given in table 3.

Highlights of this test can be summarized as follows:

1. Bird and rodent populations were relatively low on the study area and in surrounding stands. However, migrant bluebirds and robins were numerous near the end of the survey period. The short interval between seedbed preparation and sowing may have decreased small mammal populations, but freshly disked soil was probably attractive to birds.
2. Endrin levels in specimens collected before sowing were low--only 1 of 6 specimens had any detectable amount.
3. After sowing, 5 of 8 specimens contained sublethal amounts of endrin. These amounts, while judged below lethal levels, were greater than in the one specimen with endrin taken before sowing.
4. Two mice and two birds were found dead in regular surveys; endrin levels in these specimens ranged from 1.3 to 11.6 ppm. Mice contained substantially higher endrin levels than birds.

Table 3.--Endrin content of specimens collected and found dead, Wakulla R. D.

Lab Accession Number	Sample Number	Material	Parts per Million Parent	Delta Keto	Total
Specimens Collected Off Test Site Before Sowing					
7373	PR-LON-FL-1	Cotton rat	-	-	-
7373	PR-LON-FL-2	Shorttailed shrew	-	-	-
7374	PR-LON-FL-3	Bluebird	-	-	-
7375	PR-LON-FL-4	Vesper sparrow	-	-	-
7376	PR-LON-FL-5	Catbird	0.0016	-	0.0016
7377	PR-LON-FL-6	Meadowlark	-	-	-
Specimens Collected On Test Site After Last Census					
7378	PO-LON-FL-1	Robin	-	-	-
7379	PO-LON-FL-2	Robin	-	-	-
7380	PO-LON-FL-3	Mourning dove	0.1216	-	0.1216
7381	PO-LON-FL-4	Mourning dove	0.0110	-	0.0110
7384	PO-LON-FL-7	Vesper sparrow	0.1222	-	0.1222
7385	PO-LON-FL-8	Catbird	-	-	-
7382	PO-LON-FL-5	Cotton mouse	0.0228	-	0.0228
7383	PO-LON-FL-6	Cotton mouse	0.0810	-	0.0810
Specimens Found Dead On Test Site After Sowing					
7308-A	DF-1	Eastern bluebird (body)	1.648	0.037	1.685
7308-B	DF-1	Eastern bluebird (head)	0.647	0.005	0.652
7309-A	DF-2	Eastern meadowlark (body)	0.570	0.006	0.576
7309-B	DF-2	Eastern meadowlark (head)	1.092	0.002	1.094
7310-A	DF-3	Cotton mouse (body)	6.592	0.183	6.775
7310-B	DF-3	Cotton mouse (head)	0.531	0.003	0.534
7311-A	DF-4	Cotton mouse (body)	9.726	0.429	10.155
7311-B	DF-4	Cotton mouse (head)	1.433	0.055	1.488

Longleaf Pine Broadcast in Louisiana

Site description.--The site is located on the Catahoula Ranger District of the Kisatchie National Forest in Section 17, T.7 N., R.1 E. It was about 100 acres. Topography is gently rolling with an intermittent stream bisecting the area. Soils are sandy enough for moderately good internal drainage. The area was clearcut in 1971 and followed by shearing without windrowing in 1972. An excellent seedbed was established immediately prior to seeding by brush chopping 45 acres and windrowing 55 acres.

The area is irregular in shape with width ranging from 0.20 and 0.45 mile and a length of 0.65 mile. The site is bordered on one side by immature hardwood poletimber, another side by mature pine-hardwood sawtimber, and the remaining sides by immature longleaf poletimber and mature sawtimber. Ample natural food for wildlife existed in the form of dogwood berries, fruits of the American beautyberry, and a bumper crop of longleaf pine seed.

The site was sown on November 28, 1973, by aerial broadcasting about 3 pounds of longleaf (untreated weight) per acre.

Biologic surveys.--Pre-treatment bird surveys were conducted on November 19 and 20. Bird species observed on the test site, including three coveys of quail, are listed in table 4. Pre-treatment collections were conducted on November 26, 27, and the morning of the 28th. Specimens collected during this period are also shown in table 4. The rodents represent the results of 560 trap-nights.

Table 4.--Pretreatment observations and collections of birds and and small mammals

Pretreatment Observations

Mockingbird (Mimus polyglottos)
Sparrow hawk (Falco sparverius)
Junco
Sparrows
   Grasshopper
   Chipping (Spizella passerina)
   Field (Spizella pusilla)
   White-throated
   Henslow (Passerherbulus henslowii)
   Song (Melospiza melodia)
   Savannah (Passerculus sandwichensis)
Quail (#1 Covey - 10 birds)
      (#2 Covey - 13 birds)
      (#3 Covey - 12 birds)
Cardinal (Richmondena cardinalis)
Brown thrasher (Toxostoma rufum)
Wood thrush (Hylocichla mustelina)
Blue jay (Cyanocitta cristata)
Robin
Grackle (Quiscalus quiscula)
Crow (Corvus brachyrhynchos)
Warblers

Pretreatment Collections

1 House wren (Troglodytes aedon)
2 Grasshopper sparrow
1 Yellow-throated vireo (Vireo flavifrons)
1 Cardinal
2 Blue bird
1 Mourning dove
2 White-throated sparrow
4 Bobwhite quail (Colinus virginianus)
8 Harvest mouse (Reithrodontomys humulis)
3 Shorttailed shrews
4 Deer mouse (Peromyscus maniculatus)
4 Cotton rat

On November 28, 13 transects were established on the project site. Fifty marked cowbird carcasses were placed on the transect line at this time. Results of marked cowbird studies are as follows:

Test of Search Efficiency

11/29	45 marked cowbirds placed on transect lines
11/29	17 birds recovered
11/30	5 additional birds recovered (total 22)
12/2	5 additional birds recovered (total 27)
12/18	2 additional birds recovered (total 29)
	29 of 45 birds recovered

Scavenging

11/30	72 marked cowbirds placed on transects lines
12/2	64 birds recovered (3 of these had been eaten)
12/8	4 birds recovered (total 68)
	4 birds of 72 removed by scavengers

Surveys for dead or dying animals were conducted the first, second, fifth, twenty-first, and thirty-seventh day after seeding. In all, seven dead birds were found including one quail (12/3), one wood thrush (12/8), one junco (12/18), two juncos (1/13), one sparrow hawk (1/13), and one white-throated sparrow (1/13). No mice were found by search crews.

Immediately after the last search of the area, the following specimens were collected by shooting and trapping:

3 White-throated sparrow
3 Junco
1 Bluebird
4 Bobwhite quail
2 Harvest mouse
3 Deer mouse
1 Cottontail rabbit

Table 5 summarizes the results of endrin analyses of specimens collected and those found dead on the test area.

In summary, the following facts seem most important:

1. Bird and mammal populations were high. Large migrant flocks of robins, bluebirds, and blackbirds were observed flying over before and after sowing. Three coveys of quail were flushed from the area before sowing.
2. Natural food, including longleaf seed, was abundant. It is surprising that treated seed were taken.
3. Of 15 mice and birds taken before seeding, only one had a small amount of endrin.
4. After sowing, 13 of 16 specimens collected contained sublethal quantities of endrin in amounts ranging from 0.008 to 1.33 ppm.
5. Seven dead birds were found in the periodic surveys, including one sparrow hawk that had contained 3.983 ppm of endrin. Endrin levels in other specimens found dead varied from 1.06 to 6.30 ppm.
6. Longleaf seed was shattered by small birds before ingesting the endosperm. It is puzzling, then, to account for the endrin in these birds. The larger birds like quail and thrushes, probably took seed intact.

Table 5.--Endrin content of specimens collected before and after sowing, and found dead on test site, Catahoula R. D.

Lab Accession Number	Sample Number	Material	Parts per Million Parent	Delta Keto	Total
Specimens Collected Off Test Site Before Sowing					
7312	PR-LON-LA-1	Bluebird	0.014	-	0.014
7313	PR-LON-LA-2	Bluebird	-	-	-
7314	PR-LON-LA-3	Dove	-	-	-
7315	PR-LON-LA-4	White-throated sparrow	-	-	-
7316	PR-LON-LA-5	White-throated sparrow	-	-	-
7317	PR-LON-LA-6	Bobwhite quail	-	-	-
7318	PR-LON-LA-7	Bobwhite quail	-	-	-
7319	PR-LON-LA-8	Bobwhite quail	-	-	-
7320	PR-LON-LA-9	Bobwhite quail	-	-	-
7321	PR-LON-LA-10	Harvest mouse	-	-	-
7322	PR-LON-LA-11	Harvest mouse	-	-	-
7323	PR-LON-LA-12	Shorttailed shrew	-	-	-
7324	PR-LON-LA-13	Shorttailed shrew	-	-	-
7325	PR-LON-LA-14	Deer mouse	-	-	-
7326	PR-LON-LA-15	Deer mouse	-	-	-
Specimens Collected On Test Site After Last Census					
7329	PO-LON-LA-3	Junco	-	-	-
7330	PO-LON-LA-4	Junco	0.008	-	0.008
7331	PO-LON-LA-5	Junco	0.038	-	0.038
7332	PO-LON-LA-6	Bluebird	0.279	-	0.279
7335	PO-LON-LA-9	White-throated sparrow	-	-	-
7336	PO-LON-LA-10	White-throated sparrow	0.004	-	0.004
7337	PO-LON-LA-11	White-throated sparrow	0.013	-	0.013
7339	PO-LON-LA-13	Bobwhite quail	0.516	-	0.516
7340	PO-LON-LA-14	Bobwhite quail	0.192	-	0.192
7341	PO-LON-LA-15	Bobwhitequail	0.326	-	0.326
7342	PO-LON-LA-16	Bobwhite quail	1.332	-	1.332
7327	PO-LON-LA-1	Harvest mouse	0.114	-	0.114
7328	PO-LON-LA-2	Harvest mouse	0.948	-	0.948
7333	PO-LON-LA-7	Deer mouse	0.540	0.003	0.543
7334	PO-LON-LA-8	Deer mouse	0.018	-	0.018
7338	PO-LON-LA-12	Cottontail rabbit	-	-	-

Table 5. Continued

Lab Accession Number	Sample Number	Material	Parts per Million Parent	Delta Keto	Total
Specimens Found Dead On Test Site					
7301-A	DL-1	Junco (body)	0.012	-	0.012
7301-B	DL-1	Junco (head)	1.054	-	1.054
7302-A	DL-2	Bobwhite quail (body)	1.459	0.035	1.494
7302-B	DL-2	Bobwhite quail (head)	2.559	0.031	2.590
7303-A	DL-3	Woodthrush (body)	2.021	0.030	2.051
7303-B	DL-3	Woodthrush (head)	2.039	-	2.039
7304-A	DL-4	Junco (body)	3.011	0.038	3.049
7304-B	DL-4	Junco (head)	3.230	0.036	3.266
7305-A	DL-5	Junco (body)	1.249	0.014	1.263
7305-B	DL-5	Junco (head)	1.370	-	1.370
7306-A	DL-6	Sparrow hawk (body)	2.015	0.010	2.025
7306-B	DL-6	Sparrow hawk (head)	1.954	0.004	1.958
7307-A	DL-7	White-throated sparrow (body)	2.552	0.035	2.587
7307-B	DL-7	White-throated sparrow (head)	3.617	0.040	3.657

Louisiana Longleaf, Row Seeded

Site description.--This test was situated on the Evangeline Ranger District of the Kisatchie National Forest in Section 16, R.2 W., T.2 N. The area is approximately 75 acres. Soils are sandy loams characterized by fairly good internal drainage.

The site was clearcut by commercial timber sales in 1972-1973 and all stumps, logging slash and unmerchantable hardwood and pine trees were sheared and windrowed in early 1974. A wildlife pond and its associated hardwood-bordered drainage stream in the north portion of the test area were left undisturbed during these activities.

An all-weather gravel road borders the study area on the north end. Immature pine poletimber borders the area on two sides and mature longleaf pine sawtimber borders the remaining portion. Thus, the area was a small, cleared island surrounded by timbered stands, which was highly attractive to free-roaming cattle.

Seeds were sown with Hatcher front-end, single-row seeders at a rate of about 7,500 seed per acre. Sowing was done on November 4, 5, and 6, 1974. Rows were spaced approximately 8 feet apart, thus making the seed placement about every 9 inches. All of the previous test areas were broadcast sown at relatively high rates and seeds were fully exposed until they germinated or were covered by natural causes. The purpose of row seeding with the Hatcher seeder was to simultaneously cover the seeds with soil thus requiring fewer seed per acre. It was hypothesized that

the procedure would significantly minimize bird depredations, and the resulting hazard to the birds. However, a survey taken as seeding progressed showed that only 10 to 15 percent were covered with soil. Subsequent rains may have washed soil over others.

Biologic surveys.--The Louisiana Wildlife and Fisheries biologists conducted bobwhite quail censuses with trained dogs. Results were as follows:

October	25 - 1 covey, 11 birds
November	7 - 1 covey, 11 birds
November	14 - 1 covey, 11 birds
November	26 - no birds
December	3 - no birds
December	11 - no birds

The covey found before and shortly after sowing may have moved when the hunting season opened on November 28 or censusing for dead birds might have caused the covey to leave the area.

On October 23, 24, 25, 31, and November 1, the off-site, pre-treatment collections were made. Bird species observed using the test area during this time and throughout the test period are listed in table 6.

On November 4, 5, and 6, the repellent-treated seed was sown. The area was observed during this period to determine the types and relative abundance of birds feeding in the area.

On November 6, when the seeding operation was completed, 50 marked cowbirds were placed in the test area to obtain an index of the degree of scavenging of dead birds. All but one of these birds were recovered the next day.

Table 6.--Pretreatment observations and collections. Row seeding.

Pretreatment Observations

Bluebird (abundant)
White-throated sparrow (abundant)
Miscellaneous field sparrows (abundant)
Cardinal
Blue jay
Pine warbler
Brown-headed nuthatch
Carolina wren
Myrtle warbler
Yellow-throated vireo
Wood thrush
Sparrow hawk
Quail (At dawn on October 24, four coveys of quail were heard -- 8 on site and 1 just off site)
Titmouse

Pretreatment Collections, Off-Site

6 Cardinal
6 Quail
1 Crow
5 Bluebird
2 Tufted titmouse
3 Pine warbler
3 Blue jay
1 White-throated sparrow
1 Mockingbird
1 Shrike
2 Deer mouse
1 Shrew
3 Harvest mouse

Post-sowing Collection, On Test Site

3 Blue jay
5 Bluebird
3 Cardinal
2 Wood thrush
1 Carolina wren
1 Song sparrow
1 Pine warbler
1 Yellow-throated vireo
3 Cotton rat
4 Shorttailed shrew
5 Deer mouse
4 Harvest mouse

Surveys for dead and affected birds and rodents were conducted the first, second, fifth, and fourteenth day after seeding. No dead or affected animals were found. Post-treatment searches for quail on November 7 and 14 using trained dogs yielded 1 covey of quail remaining on the area. Three later searches failed to locate any quail in the test area.

Bird and mammal collections are shown in table 6. Small mammals taken are the result of 146 trap nights before sowing and 240 after sowing. Results of the endrin analysis are listed in Table 7.

Important highlights of this test were:

1. Site was prepared about 4 months before sowing, leaving only short grass cover on the area. As a small opening in a large timbered area, it was probably highly attractive to birds.

2. A wide variety of birds, including one quail covey, was on or near the area in reasonably large numbers. The mouse population was about average.

3. Natural foods in the general area were reasonably available.

4. Eleven of the 19 bird and mammal specimens taken before sowing contained sublethal doses of endrin. Nine of the 13 birds collected contained endrin.

5. Only 8 of 19 specimens collected after surveys were completed contained endrin; all endrin levels were sublethal. Only 50 percent of the bird specimens had endrin.

6. Although the area was easy to search, no dead birds or mammals were found in repeated, intensive surveys.

Table 7.--Endrin content of birds and mammals taken before and after sowing. Row seeding.

Lab Accession Number	Sample Number	Material	Parts per Million 1/ Total Endrin
Specimens Collected Off Test Site Before Sowing			
7386	PR-LA-74-1	Cardinal	0.004
7387	PR-LA-74-2	Cardinal	0.006
7388	PR-LA-74-3	Cardinal	--
7389	PR-LA-74-4	Quail	0.021
7390	PR-LA-74-5	Quail	--
7391	PR-LA-74-6	Quail	0.002
7392	PR-LA-74-7	Bluebird	0.010
7393	PR-LA-74-8	Bluebird	0.003
7394	PR-LA-74-9	Bluebird	0.011
7395	PR-LA-74-10	Blue jay	--
7396	PR-LA-74-11	Blue jay	0.003
7397	PR-LA-74-12	Blue jay	--
7398	PR-LA-74-13	White-throated sparrow	0.017
7399	PR-LA-74-14	Deer mouse	--
7400	PR-LA-74-15	Deer mouse	--
7401	PR-LA-74-16	Shrew	0.003
7402	PR-LA-74-17	Harvest mouse	--
7403	PR-LA-74-18	Harvest mouse	--
7404	PR-LA-74-19	Harvest mouse	0.003
Specimens Collected On Test Site After East Census			
7405	PO-LA-74-1	Blue jay	--
7406	PO-LA-74-2	Blue jay	--
7407	PO-LA-74-3	Blue jay	0.013
7408	PO-LA-74-4	Bluebird	0.002
7409	PO-LA-74-5	Bluebird	0.002
7410	PO-LA-74-6	Bluebird	0.029
7411	PO-LA-74-7	Cardinal	--
7412	PO-LA-74-8	Cardinal	--
7413	PO-LA-74-9	Cardinal	0.010
7414	PO-LA-74-10	Wood thrush	--
7415	PO-LA-74-11	Wood thrush	--
7416	PO-LA-74-12	Song sparrow	0.009
7417	PO-LA-74-13	Shrew	--
7418	PO-LA-74-14	Shrew	0.057
7419	PO-LA-74-15	Deer mouse	--
7420	PO-LA-74-16	Deer mouse	0.016
7421	PO-LA-74-17	Harvest mouse	--
7422	PO-LA-74-18	Harvest mouse	--
7423	PO-LA-74-19	Harvest mouse	--

1/ No parent or Delta Keto endrin detected.

Broadcast Loblolly in Louisiana

Site description.--The site is located on the Evangeline Ranger District of the Kisatchie National Forest in Section 13, T.2 N, R.3 W. It is approximately 100 acres. Topography is gently rolling. Soils are primarily Ruston, Bowie, and Orangeburg soils which are fairly well drained.

The site was prepared for loblolly seeding by shearing and burning several months prior to seeding. The seedbed was judged to be excellent.

The area is irregular in shape with the width ranging from 0.1 to 0.5 mile wide and 0.6 mile long. It was bordered on two sides by roads (one dirt and one all-weather) and on two sides by longleaf pine stands. Basically it is a cleared area surrounded by timbered stands, presenting an attractive site to birds.

Sowing.--Seeds were sown by helicopter on January 30, 1974 at the rate of approximately 1 pound (untreated weight) of seed per acre.

Biologic surveys.--On January 28, 29, and 30 pre-treatment bird and mammal collections were made several miles from the test site. Collected specimens are listed in Table 8 along with a listing of bird species observed using the test area during this time. Small mammals taken were the results of 438 trap nights before sowing and 211 trap nights after sowing.

Table 8.--Pretreatment observation and collections of birds and mammals. Evangeline R.D.

Pretreatment Observations

Bluebird - abundant
Goldfinch - common
Myrtle warbler - common
Mockingbird
Blue jay
Sparrows - various species
Warblers - various species
Crow
Cardinal
Robin
Quail

Bird and Mammal Collections Made Off-Site Before Sowing

10 Bobwhite quail
10 Harvest mouse
3 Junco
7 Bluebird
4 White-throated sparrow
2 Eastern meadowlark
3 Deer mouse
2 Shorttailed shrew

On January 30, when the endrin-treated seeds were applied by helicopter, 50 marked cowbirds were placed in the test area to obtain an index of the degree of scavenging of dead birds. Surveys for dead or dying animals were conducted the first, second, fifth, twenty-first and thirty-sixth day after seeding. Results are given below:

Test of Search Efficiency

1/31/74	50 marked cowbird carcasses randomly placed on 18 transect lines
1/31/74	22 marked birds recovered
2/1/74	9 marked birds recovered (total 31)
2/4/74	3 marked birds recovered (total 34)
	34 of 50 birds recovered

Scavenging

2/1/74	54 marked cowbird carcasses placed on transect lines 1 bird was placed in the field, 1 bird was placed in each end of the transect lines in the edge of the woods
2/4/74	47 marked birds recovered
	7 birds of 54 were lost to scavenging

A small pond located in the test area was checked during each post-seeding inspection. Checking of the pond consisted of walking the perimeter and searching for dead or dying amphibia, crustaceans or fish. Crawfish and several fin fish were netted on the last inspection. Several frogs were observed but not captured. Several specimens, listed in table 9, were analyzed for endrin.

Specimens were later delivered to the Environmental Monitoring Laboratory in Gulfport, Mississippi. Results of the endrin analysis are given in table 9.

Table 9.--Endrin content in specimens collected before and after sowing

Lab Accession Number	Sample number	Material	Parts per Million Parent	Delta	Keto	Total
Specimens Collected Off Test Site Before Sowing						
7344	PR-LOB-LA-2	Bob white quail	--	--		--
7347	PR-LOB-LA-5	Junco	--	--		--
7348	PR-LOB-LA-6	Junco	--	--		--
7351	PR-LOB-LA-9	Bluebird	0.016	--		0.016
7352	PR-LOB-LA-10	Bluebird	0.005	--		0.005
7353	PR-LOB-LA-11	White-throated sparrow	--	--		--
7354	PR-LOB-LA-12	White-throated sparrow	--	--		--
7355	PR-LOB-LA-13	Eastern meadowlark	--	--		--
7356	PR-LOB-LA-14	Eastern meadowlark	--	--		--
7345	PR-LOB-LA-3	Harvest mouse	--	--		--
7346	PR-LOB-LA-4	Harvest mouse	--	--		--
7349	PR-LOB-LA-7	Shorttailed shrew	--	--		--
7350	PR-LOB-LA-8	Shorttailed shrew	--	--		--
7357	PR-LOB-LA-15	Deer mouse	--	--		--
7358	PR-LOB-LA-16	Deer mouse	--	--		--
Specimens Collected On Test Site After Last Census						
7343	PO-LOB-LA-1	Bob white quail	--	--		--
7359	PO-LOB-LA-1&2	Sunfish	--	--		--
7360	PO-LOB-LA-3&4	Mosquito fish	0.010	--		0.010
7361	PO-LOB-LA-5	Bluebird	0.394	--		0.394
7362	PO-LOB-LA-6	Bluebird	0.178	--		0.178
7363	PO-LOB-LA-7	Myrtle warbler	0.006	--		0.006
7364	PO-LOB-LA-8	Myrtle warbler	--	--		--
7367	PO-LOB-LA-11	Chipping sparrow	--	--		--
7368	PO-LOB-LA-12	Chipping sparrow	--	--		--
7369	PO-LOB-LA-13	Sparrow hawk	0.139	--		0.139
7365	PO-LOB-LA-9	Harvest mouse	0.713	0.012		0.725
7366	PO-LOB-LA-10	Harvest mouse	1.311	0.005		1.316
7370	PO-LOB-LA-14	Shorttailed shrew	--	--		--
7371	PO-LOB-LA-15	Shorttailed shrew	--	--		--

Highlights of this test can be summarized as follows:

1. The area was probably very attractive to birds as it was an opening surrounded by pine and hardwood stands.

2. Birds, especially bluebirds, were numerous. Mice and shrews were also numerous. Consequently, there was potentially heavy predation pressure on the area.

3. Only two specimens of the 16 birds and mammals collected before sowing had detectable quantities of endrin--both were bluebirds.

4. Six of 11 bird and rodent specimens taken after the last survey contained sublethal levels of endrin, ranging from 0.006 to 0.725 ppm.

5. A very small amount of endrin was found in one of the two fish samples.

6. No dead birds or mammals were found in intensive searches of the area, although loblolly seed is small enough to be ingested totally by even the smallest seed-eating birds.

DISCUSSION

The four tests reported in previous sections raised about as many questions as they resolved.

In three of the four tests, the percentage of birds taken after the last census that contained endrin was higher than in presowing collections. In the other test, the trend was reversed. Overall, 36 percent of the birds collected before sowing had measurable quantities of endrin as contrasted to 72 percent of those taken after the last census. Only one bird, a quail, of those taken after the last census contained more than 1 ppm endrin, and only three birds contained over 0.5 ppm. When the same species were represented in both collections, the endrin contents changed as shown below:

Species	Total endrin content--ppm	
	Before sowing	After censusing
Vesper sparrow	0	0.122
Catbird	0.002	0
Bluebird	.014	.279
Bluebird	0	--
White-throated sparrow	0	.004
White-throated sparrow	0	.013
Bobwhite quail	0	.516
Bobwhite quail	0	.192
Bobwhite quail	0	.326
Bobwhite quail	0	1.332
Cardinal	.004	0
Cardinal	.005	0
Bluebird	.010	.001
Bluebird	.003	.002
Bluebird	.011	.029
Cardinal	0	.010
Blue jay	0	--
Blue jay	.003	--
Blue jay	0	.013

As a whole, rodents contained more endrin than birds, especially those taken after the last census. This is understandable as mice and rats hull seeds and in doing so are exposed to endrin. A very small percentage of caged mice eat as many as 10 seeds, and they are usually killed. The big majority, however, eat a few seeds and reject treated seeds thereafter. In both cases, it is logical to find some endrin in specimens.

Dead specimens found on the test areas in systematic searches varied widely in numbers. Dead birds were found on two areas and no kills were detected on the other two areas. Strangely enough, the highest bird kill occurred on a site that seemed to present the least hazard. While much of the area had very little vegetation to conceal seeds, natural food including longleaf seed was very abundant.

As previously mentioned, kill of small birds with treated longleaf is puzzling because they must shatter the relatively large seeds to obtain the endosperm, in contrast to larger birds that eat the seed intact. Presumably, some endrin gets in their mouths when holding a seed in their beaks and when pecking at the seed to break it open.

The highest bird kill was on the Catahoula R. D. The seven birds recovered may equate to roughly 3/4 bird per acre when it is considered only 20 percent of the area was searched, recovery rates of birds placed on the area were low, and predators took some carcasses.

Unfortunately, no estimates of bird visits to test sites were obtained. Needless to say this is a difficult parameter to estimate. We only know that bird populations were "low" in Florida and sightings were "high" in the Louisiana tests. Moreover, all areas were attractive to birds because of recent soil disturbance or burning.

The sparrow hawk found dead on the Catahoula site may have been due to secondary poisoning. Certainly, a few rodent specimens taken contained sufficient endrin to lead to secondary poisoning. However, specimens of this species were not collected before sowing so initial endrin levels were not established. There remains, then, the possibility that the toxicant was obtained in nearby cotton fields where endrin is used six to eight times yearly.

The row seeding on the Evangeline Ranger District failed to give the desired test. It was designed to determine if covering the seed with soil during the sowing operation would lessen danger of birdkill. However, only 10 percent of the seeds were covered. Despite the failure to conceal most of the seeds, no kill was detected in the surveys.

The great variation in kills and endrin levels of specimens taken suggest more trials are needed if a better estimate of hazards to wildlife are needed. This can be costly and endless because many factors such as climate, alternate food sources, numbers of birds and mammals, cover on the site, and migrations can affect the outcome. The tests have established that a few birds exhibited acute levels of endrin while a higher number exhibited chronic levels of endrin. This alone dictates some safety precautions are needed. What are they? The following recommendations are designed to reduce the exposure to bird populations:

First, endrin should not be used unless precensusing or experience indicates mammal populations are high enough to warrant the use of a repellent. Four or five animals per acre will probably justify inclusion of endrin in the repellent coating. The rationale for this is that harvest and white-footed mice ingest about 100 seeds daily in the laboratory when other food is unavailable. Since both species are sown at rates averaging about 12,000 seeds per acre and field germination normally extends for 60 to 80 days, it is obvious that a relatively low small mammal population can cause heavy seed losses if repellents are not used. Longleaf seeds are much larger than loblolly and slash so daily losses from mice are smaller. But sowing rates with longleaf are also lower, which offsets the advantage of the larger seed.

Second, longleaf seed should be sown on a 1-year grass rough in preference to a fresh burn, disked, or cleared area with high exposure of mineral soil. Better germination will be obtained as the grass helps preserve moisture in the upper soil layers by shielding from the sun and desiccating winds. A light grass cover will also be less attractive to birds than a fresh burn or newly disked soil. It will, however, provide a better habitat for small, seed-eating mammals, but of all the choices the light rough is the best.

Third, on open sites with a heavy grass sod, it is recommended that loblolly and slash pine be sown on disked strips to boost first-year survival and early growth. It will also lessen the hazards to birds as many seeds are covered by soil during rains. If mechanical preparation is not employed, the preferred seedbed is a light grass rough for reasons given in the preceding paragraph. On hardwood sites where the grass is sparse, a light

cover of leaves should be retained by burning before all leaves have fallen. Not only does the mantle of leaves aid germination, but many seeds wash beneath the leaves and are concealed from predators.

Fourth, sow seed only when conditions are favorable for prompt germination. Long exposure of seed waiting for favorable germination conditions results in an unnecessary hazard to wildlife.

Fifth, a 100-foot-wide unseeded buffer strip should be left around the perimeter of any field seeded with endrin-treated seeds. This would reduce the exposure to many of the bird species which concentrate along the edge of woods. It might also reduce the attraction of rodents into the seeded area.

And, finally, maximum use should be made of row seeding that covers seed with soil.

FUTURE PLANS

The study is closed with this report.

It is recommended that results of the four tests be published jointly by personnel of the U. S. Forest Service and the U. S. Fish and Wildlife Service. Tuttle should decide who will have major responsibility for the first draft, the authors, and the outlet.

APPENDIX

SAMPLING AND ANALYSIS OF PESTICIDES IN THE ENVIRONMENT

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INTRODUCTION

Pesticides are intentionally released into the environment to protect the health and welfare of the nation. Because of their benefits, pesticides have become increasingly important to agricultural production, to public health and sanitation, to protect capital investment and natural resources, and to improve human comfort and well-being.

Since pesticides are biological poisons, in most cases, (some are only sensory modality effectors — repellents and attractants) their proliferation in the environment is causing some concern for human health and for their effect on other components of the environment.

Because of this concern, the Federal Insecticide, Fungicide, and Rodenticides Act (FIFRA) was amended in 1972, imposing new regulations for controlling so-called "unreasonable adverse effects" on the environment by pesticides. Section 20(c) of the FIFRA requires monitoring activities. Other legislation, such as the Water Pollution Control Act and the National Environmental Policy Act, requires that Federal agencies "monitor, evaluate, and control on a continuing basis their agencies' activities so as to protect the environment."

In an effort to measure the amount of environmental contamination directly related to applied pesticides, monitoring programs have been established. Any effective monitoring program must be designed to correlate pesticide residue levels with any discreet and gross changes in naturally occurring animal populations. Furthermore, a program of good biometric design must be employed, which necessitates sampling a variety of environmental components. These components include soil, water, sediment, air, vegetation, fish and wildlife.

Pesticides are subject to breakdown in the environment as a result of hydrolysis and/or oxidation, photodecomposition, metabolism, and microbial degradation. These mechanisms produce by-products from the parent compounds, which must also be monitored at part per million and part per billion levels, as well as the parent compounds. To successfully attain these measurable levels, utilizing small sample weights, it is necessary to have methodology that is sensitive and specific.

Gas-liquid chromatography (GLC), primarily, along with column and thin-layer chromatography, is used to detect pesticide residues at nanogram and picogram levels. Methods of confirmation are employed, such as mass spectrometry (1), photochemical conversion (2), p-values (3), derivatization (4), and specific detectors (5) other than the electron capture detector.

The electron capture detector is used in the analysis of organochlorine and organophosphorus pesticides. This detector has sensitivity in the sub-picogram levels. The Linear Nickel-63 Electron Capture Detector has replaced, almost entirely, the electron capture detector with the tritium source. The Nickel-63 Detector has a linear response factor of from 1 to 10,000 within 10% (6), can be operated at higher temperatures (300-350°C), and requires very little maintenance.

The organophosphorus pesticides are also detected the flame-photometric and thermionic detectors (7) at nanogram and picogram levels. Specificity for organonitrogen pesticides is obtained using thermionic and electrolytic conductivity detectors at nanogram and picogram levels. The microcoulometric detector (8) is specific for chlorine, sulfur, nitrogen, and phosphorus containing pesticides with sensitivity at nanogram levels.

SAMPLING

In monitoring large pesticide programs, a good biometrical design must be employed in order that a meaningful interpretation of the data obtained may be made. In monitoring the Imported Fire Ant Program, for example, a sampling site of ¼ acre (105 ft x 105 ft) is selected on a best of fit basis from 5 randomly selected sites within each 20,000 acre sampling area. These samples are environmental components which would be representative of the area being sampled. To establish population trends in animals and environmental impact, components such as soil, sediment, water, invertebrates, fish, birds, mammals, air, and vegetation are collected from each site for pesticide residue analysis

Before samples are taken, all the equipment used must be scrupulously cleaned with water, followed by isopropyl alcohol, and allowed to air dry. The vehicles used to transport the samples must be cleaned thoroughly and should never be used to haul pesticides.

Soil samples are collected with a hand operated auger, which takes cores 1 in. to 3 in. in depth and 3 in. in diameter. On a sampling site, sampling begins 7½ ft. from the border of the site. A soil core is collected every 15 ft. until 7 cores are collected; then at a point 15 ft. perpendicular to this line of sampling, another sample is taken and the process repeated on a line parallel to the previous line of sampling. A total of 49 soil cores is collected, sieved through a hardware cloth screen into a 3 gallon galvanized pail, and thoroughly mixed. The sample is then transferred to two ½ gallon cans with lids, along with a sample data form attached to the outside of one of the cans. This form is coded and contains information, such as date sample was collected, site number, county, state, time sample was collected, type of sample, etc. The cans and forms are shipped to the laboratory along with other types of

samples collected from the site for pesticide residue analysis.

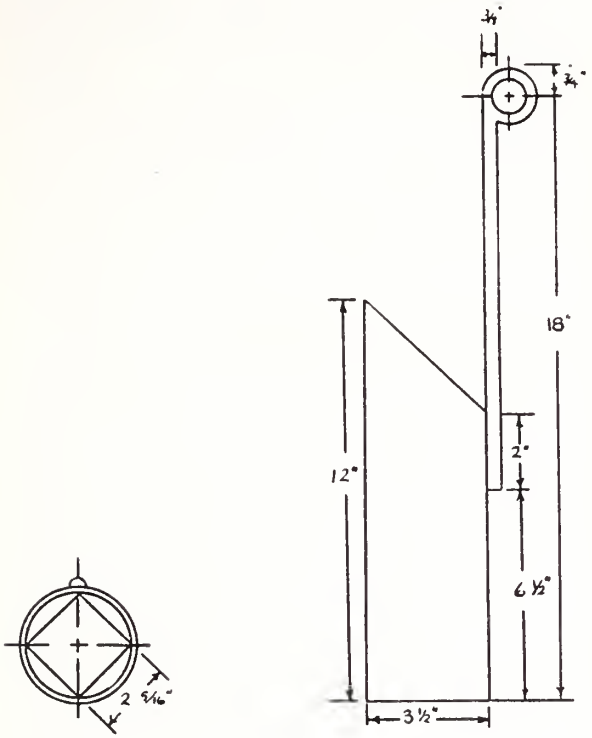

Figure 1.

Dredge-type device for collecting sediment samples.

Sediment samples are collected with a dredge (Figure 1), which is thrown at least ten times in the water source of the site to collect samples. Each time the dredge sample is collected, it is transferred to a 3 gallon galvanized pail, and the same procedure is followed as with the soil samples. A hole is made in the lid of the can of each sediment sample to release any gas build-up from organic matter in the sample.

Water samples are collected in gallon jugs immersed below the water surface at several points. Care should be exercised that bottom sediment is not disturbed. One gallon of water is collected from each site, and the sample data form is attached, as described in the section on soil samples.

Invertebrate samples are collected in pitfall traps, as described by Wojick, et al. (9). These samples are separated according to genus and species and wrapped in aluminum foil with the shiny side of the foil being outside. The sample is tagged and the information concerning the sample is recorded on the tag and the sample data form is also attached for shipment to the laboratory.

Fish samples are collected by fish traps, seines, or conventional fishing equipment from the water source of the sampling site. These samples are separated into species and wrapped in aluminum foil, tagged, and placed in plastic bags with the sample data form and shipped to the laboratory for analysis.

Bird samples are frequently collected using Japanese mist nets. These nets are placed near the water source or in a cove where the net is not visible. After collection, the birds are separated into species and treated the same as the fish in regard to wrapping, labelling and shipping.

Mammal traps are employed for sampling mammals; these traps are baited with peanut butter, or some other foodstuff, and have a trap door that closes when the animal tries to retrieve the food. The Sherman Mammal Trap is used to capture animals no larger than a Norway Rat. Larger traps of various designs are employed to capture larger mammals, such as raccoons, opossums, rabbits, etc. These animals are separated into genus and species, wrapped in aluminum foil, placed in a plastic bag, labeled, and shipped to the laboratory for analysis.

Air samples can be collected using air sampling machines which contain four impingers in series connected to a vacuum pump. The air is sucked into the impingers, which contain ethylene glycol or some other solvent with low volatility. The total amount of air sampled (m^3) is controlled by means of a flow meter and timer. The samples of ambient air extracts and the filters used in the machine to collect particulate matter are shipped to the laboratory in glass containers with labels and the sample data forms. Good air sampling machines are still much in question. Some agencies have suspended their air monitoring programs until a reliable method is achieved.

Vegetation samples are collected from the same sampling area as the soil samples. These samples are taken with shears, sickles, pocket knives, etc. These samples should be placed in cloth bags and covered with a plastic bag. They are then labeled, and a sample data form accompanies the sample to the laboratory, as described previously.

All samples that are perishable are frozen and shipped to the laboratory in styrofoam containers with dry ice.

PROCEDURE FOR SYSTEMATICALLY RECEIVING, NUMBERING, AND STORING ENVIRONMENTAL COMPONENTS

Samples and paper work received at the Pesticide Monitoring Laboratory are handled in the following manner.

The originals of the sample data forms are forwarded to the laboratory from the field as soon as possible after collection of the sample. Upon arrival at the laboratory, they are stamped with the date received, separated according to component type (soil, sediment, mammals, birds, etc.), and then placed in order according to state and numerical site number. A separate log of type samples from each site number and the number of each indicator are recorded in the site log book.

The site log book lists in numerical order the site numbers assigned to each state and the number of samples collected from each site. This book is used to check against the number of samples when they arrive at the laboratory and to ascertain if all samples have been completed after analysis.

A separate Laboratory Accession Number Book is maintained and numbers are assigned to each sample data form. Laboratory accession numbers are derived by taking the last digit of the fiscal year the sample was collected, that is, fiscal year 1974 would have the first digit of 4; fiscal year 1975 would be 5, and then adding zeros to make a six digit number. Fiscal year 1975 samples' first number would be 500,001 and on. The

laboratory accession number is stamped on the original of the sample data form in the upper right hand corner and recorded in the Laboratory Accession Number Log Book by accession number, type of sample (using code), state, county, site, number, and remarks. Under "remarks," information pertaining to the sample is recorded.

The original sample data forms are maintained on file by numerical accession laboratory numbers until the sample has been completely processed for analysis.

A complete record of biological type samples is maintained for each individual state in which the sample was collected. This is necessary to fulfill collection permit requirements. The biological code number of the species, the common name of the species, the scientific name, and the total number of the species collected are listed in this record.

Upon arrival of samples to the laboratory, they are checked for damage in shipment, spoilage, etc. Soil and sediment samples are stacked according to site numbers and states until the laboratory accession numbers are assigned. Water and/or vegetation samples are stored under refrigeration. The carbon copy of the sample data form is attached to each individual sample and is compared to the information recorded on each sample for positive identification of that sample. The carbon copy of the sample data form is then removed from the sample, compared with the original of the sample data form and the Laboratory Accession Number Log Book, and then assigned the proper laboratory

Upon arrival at the laboratory, samples are checked for damage in shipment, spoilage, etc. Soil and sediment samples are stacked according to site numbers and states until the laboratory accession numbers are assigned. Water and/or vegetation samples are stored under refrigeration. The carbon copy of the sample data form is attached to each individual sample and is compared to the information recorded on each sample for positive identification of that sample. The carbon copy of the sample data form is then removed from the sample, compared with the original of the sample data form and the Laboratory Accession Number Log Book, and then assigned the proper laboratory number.

The carbon copy is then used to insure that the sample was received and marked with the laboratory number and corresponds with the number in the Accession Log Book, and is then filed by site number in an individual site number folder. After analysis, the carbon copy is attached to the chromatograms and filed by site number.

Samples are processed by groups, and group letters are used to identify each group. This system simplifies locating samples for re-check, and in the event of a contamination, prevents having to rerun large numbers of samples.

Retention samples and their extracts are retained in freezers and refrigerators until after the analyses are completed.

EXTRACTION AND CLEANUP

Soil

The moistened sample is placed in a ½ gallon canning jar with a 2:1 solvent-sample ratio (v:w). The solvent employed is a 3:1 hexane-acetone mixture. The jar is sealed with a Teflon liner, and the sample shaken for four hours. A suitable aliquot is decanted, and the acetone removed by washing with distilled water in a separatory funnel. This hexane extract is filtered through anhydrous sodium sulfate, and the volume reduced on a hot plate to a final volume of 50 ml prior to cleanup and analysis.

Sediment

The extraction of sediment samples is identical to that of soil samples except that no water is added initially to moisten the sample.

With soil and sediment samples, weighed portions are placed in an oven at 100°C for eight hours and then reweighed in order to determine the moisture content. These "dry weights" are used in the final calculation of pesticide residue levels.

Water

The water sample is shaken in the sampling container and then poured into a separatory funnel before the particulate matter present can settle. The sample is extracted three times with methylene chloride, the sample to solvent ratio being 10:1 (v:v). The extracts are filtered through anhydrous sodium sulfate, a Nujol keeper added, and the volume reduced to approximately 5 ml; 50 ml of hexane are added and the volume again reduced. This procedure is repeated once more to remove all traces of methylene chloride, and the volume adjusted to a final volume of 10 ml in hexane.

Invertebrates

The sample is weighed and ground in a mortar along with enough anhydrous sodium sulfate to absorb the water in the sample. The sample is placed in a canning jar and the mortar and pestle rinsed three times with 50 ml portions of hexane. These washings are added to the canning jar, the sample shaken for two hours, and the volume then reduced on a hot plate to a final volume of 10 ml.

Fish, Birds, and Mammals

These samples are initially ground with a hand grinder or a Hobart® chopper. A suitable portion is placed in a canning jar and a solvent system of 3:1 hexane-isopropyl alcohol added in a 20:1 (v:w) solvent to sample ratio. The sample is shaken for two hours and a suitable aliquot decanted into a separatory funnel. The isopropyl alcohol is washed out by two successive washings with saturated sodium chloride solutions, followed by washing with distilled water. The hexane extract is then filtered through anhydrous sodium sulfate, and the volume reduced on a hot plate to a final volume of 45 ml prior to cleanup and analysis.

Air

The ethylene glycol from the impingers is placed in a half gallon canning jar, along with equal amounts of distilled water and hexane. The glass sampling train, impingers, filters, and glass wool are washed with hexane and the washings added to the extraction mixture. After the mixture has been shaken for two hours, it is transferred to a separatory funnel and the bottom layer discarded. The hexane layer is washed with distilled water, the water layer discarded, and the hexane extract filtered through anhydrous sodium sulfate. The volume is then reduced to 10 ml prior to cleanup and analysis.

Vegetation [Less than 2% oil content]

The sample is placed in a four-liter stainless steel blender and blended for three minutes with a 3:1 hexane:acetone mixture, the solvent to sample ratio being 10:1 (v:w). A suitable aliquot is decanted into a separatory funnel and the acetone removed by washing with distilled water. The hexane extract is filtered through anhydrous sodium sulfate and the volume adjusted to 50 ml on a hot plate prior to cleanup and analysis.

Vegetation [greater than 2% oil content]

If a systematic investigation is being pursued, the grain is washed with isopropanol and hexane to remove surface pesticides.

The sample is ground to a fine powder in a blender and isopropyl alcohol added in a solvent to sample ratio of 1:1 (v:w). The sample is blended for one minute, and the contents transferred to a canning jar with a measured amount of hexane — usually a solvent to sample ratio of 3:1 (v:w). The mixture is shaken for two hours, and a suitable aliquot then removed and placed in a separatory funnel. The alcohol is removed by two successive washings with a saturated sodium chloride solution. The hexane extract is washed a final time with distilled water and then filtered through anhydrous sodium sulfate. The volume is then reduced to 50 ml on a hot plate prior to cleanup and analysis.

Florisil Column

Sample cleanup is accomplished by fractionation on an 11 x 500 mm column packed with 15 g of 60-120 mesh Florisil. The Florisil is pre-washed with 100 ml of methylene chloride before the sample is added. The sample extract is eluted with 150 ml of a 5% (v:v) methylene chloride: hexane solution followed by elution with 100 ml of methylene chloride. Mirex, heptachlor, p,p'-DDT and its metabolites are located in the first fraction, while dieldrin, endrin, and organophosphorus compounds are contained in the second fraction. Heptachlor epoxide may appear in either fraction depending on the particular batch of Florisil.

Acetonitrile Partitioning

A measured aliquot of the hexane sample extract is increased in volume to 50 ml and placed in a separatory funnel with 100 ml of hexane-saturated acetonitrile. The mixture is shaken and the bottom layer removed and retained. The upper layer is similarly extracted twice more and the lower layer acetonitrile extracts combined. To this acetonitrile mixture is added 20 ml of acetonitrile-saturated hexane in a separatory funnel. The mixture is shaken and the lower level collected. The volume is reduced on a hot plate to approximately 10 ml; 100 ml of hexane are added and the volume again reduced to 10 ml. This process is repeated once again to remove all traces of acetonitrile, and the volume adjusted to 15 ml prior to column chromatographic cleanup.

Silicic Acid Column

When polychlorinated biphenyls (PCBs) are present as interfering substances in samples, it is necessary to resort to the method of Armour and Burke (10) in order to separate PCBs from pesticides.

ANALYSIS

The gas-liquid chromatograph (GLC) is used to analyze the samples after extraction and cleanup. To effectively analyze a large number of samples in a relatively short time frame, a totally automated gas-liquid chromatography system is employed in our laboratory. This entire system is composed of eight gas-liquid chromatographs, each equipped with automatic sample injectors, analog to digital converters, strip-chart recorders, linear nickel-63 detectors, and two teletypewriters used to input data and to give presentations of the final analyses.

The controller is programmed by a punch tape which is fed into the controller's memory by a high-speed tape reader. After the program has been entered, the controller is digitized, calibrated, and the external standard introduced.

The external standard contained the organochlorine pesticides most commonly found in the environment. Each sample and external standard is injected onto two ypes of packed columns. Figures 2 and 3 exhibit the types of columns used in the analyses of these environmental samples, along with the operating parameters. The two column system is one of the methods used to confirm the identities of residues that are found.

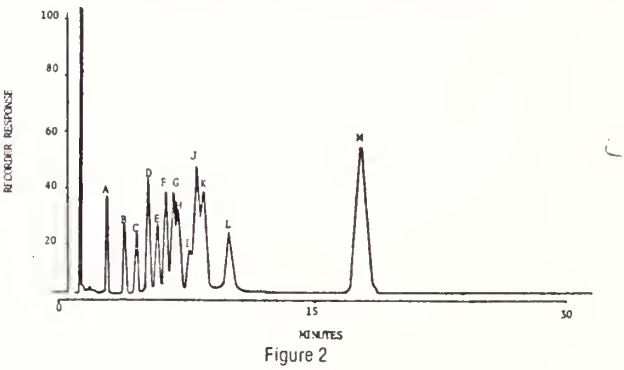

Figure 2

Electron-capture gas chromatogram of A. lindane; B. heptachlor; C. aldrin; D. heptachlor epoxide, E. o,p'-DDE; F. gamma-chlordane; G. p,p'-DDE; H. dieldrin; I. endrin; J. p,p'-TDE; K. o,p'-DDT; L. p,p'-DDT; M. mirex. Six foot glass column (¼ in. o.d.) of 10% DC-200 on Gas Chrom Q (100-120 mesh). Temperatures: Injector 250°C; oven 230°C; detector, 300°C. Gas flow. 37.5 ml/min of argon (5% methane). Recorder attenuator = 32.

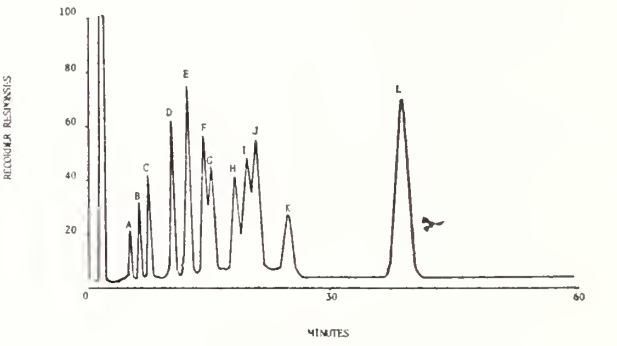

Figure 3

Electron-capture gas chromatogram of A. lindane; B. heptachlor, C. aldrin; D. heptachlor epoxide; E. gamma-chlordane and o,p'-DDE; F. p,p'-DDE; G. dieldrin; H. endrin; I. o,p'-DDT; J. p,p'-TDE; K. p,p'-DDT; L. mirex. Six foot glass column (¼ in. o.d.) of 1.5%/1.95% OV-17/QF-1 on Gas Chrom Q (100-120 mesh). Temperatures: injector 250°C; oven 220°C; detector 300°C Gas Flow. 14 ml/min of argon (5% methane). Recorder Attenuator = 128

After each analysis is completed, a print-out is obtained from the output teletypewriter describing the type of sample, name of pesticides present in parts per million, and the number of the gas-liquid chromatograph which analyzed the sample.

A sample being analyzed for organophosphorous pesticides may be injected onto a gas chromatograph equipped with either a flame photometric or a thermionic detector. Nitrogen-containing samples, such as the triazine herbicides, may be analyzed using a thermionic detector. The amounts of pesticides present in the samples are calculated by comparing peak heights to those of standards.

The name of the pesticide and its residue in parts per million are recorded on the sample data form and its carbon copy which is accompanied by the sample from the field. The carbon copies and the chromatograms and computer print-outs are attached and filed. The original of the sample data form is key punched, and the punched card is forwarded to a central data center to be incorporated into a special program. This special program is designed to correlate the residue and field data.

CONCLUSION

Environmental monitoring is now recognized as essential in areas of pesticide use in order to assess the impact of these pesticides on the environment. During the last two decades, advances in instrumentation in terms of detection limits and specificity, along with the growing expertise of residue chemists, have made reports of pesticide residue levels in the parts per billion range commonplace. Using this technical ability and carefully assessing the significance of the data obtained, pesticides can be used in a more efficacious manner with less possible damage to the environment. □

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Biologicals (weighing 20 g or more)

Biological samples are initially ground with a Hobart chopper or a hand grinder. A 20 g portion of the ground sample is placed in a quart canning jar; and 100 ml isopropyl alcohol plus 300 ml nanograde hexane are added. The jar is then sealed using a Teflon liner and placed on a shaker for two hours. A 300 ml aliquot (which represents 15 g of sample) is decanted into a 500 ml graduate cylinder and then into a 500 ml separatory funnel. The isopropyl alcohol is removed by two successive washings with 100 ml portions of a saturated NaCl solution, followed by washing with distilled water, the washings being discarded. In order to avoid the formation of troublesome emulsions during these washings, the separatory funnel should be swirled rather than shaken vigorously. The remaining hexane extract is filtered through a filter funnel containing a plug of pre-washed glass wool and 1 1/2 inches of anhydrous sodium sulfate into a 500 ml 24/40 S Erlenmeyer flask. One ml of an 0.01% v/v Nujol solution is added as a keeper: a few glass beads are added: and a three-ball Snyder column is attached to the flask. The solution is then concentrated in the hood on an explosion-proof hot plate to approximately 25 ml, and then quantitatively transferred with washing into a 50 ml graduate cylinder. The volume is adjusted to 45 ml, and the sample is placed in a 50 ml culture tube prior to cleanup and analysis.

Biologicals (weighing less than 20 g)

The sample is weighed and placed in a mortar with enough anhydrous Na_2SO_4 added to absorb the moisture in the sample. The sample is ground with a pestle and placed in a quart fruit jar. The mortar and pestle are rinsed three times with 50 ml portions of hexane and the washings placed in the fruit jar with the sample. The sample is placed on a shaker for two hours and the extract decanted. This extract is then placed on the column for cleanup (See cleanup procedures).

CLEANUP PROCEDURES

U

Florisil Column Cleanup

The apparatus employed is a 125 ml reservoir with an 11 x 500 mm glass column having a removable Teflon stopcock assembly and a detachable glass tip (Fig. 1). A small piece of pre-washed glass wool is placed in the bottom of the column and approximately 1/2 inch of anhydrous sodium sulfate is added. 15 g of 60-120 mesh Florisil are placed in the column and tapped lightly to insure even packing. Another inch of anhydrous sodium sulfate is then added to the top of the Florisil, and the column washed with 100 ml of methylene chloride by allowing the level of CH_2Cl_2 to drain down to the top of the sodium sulfate. Care must be exercised that the column not be allowed to go dry.

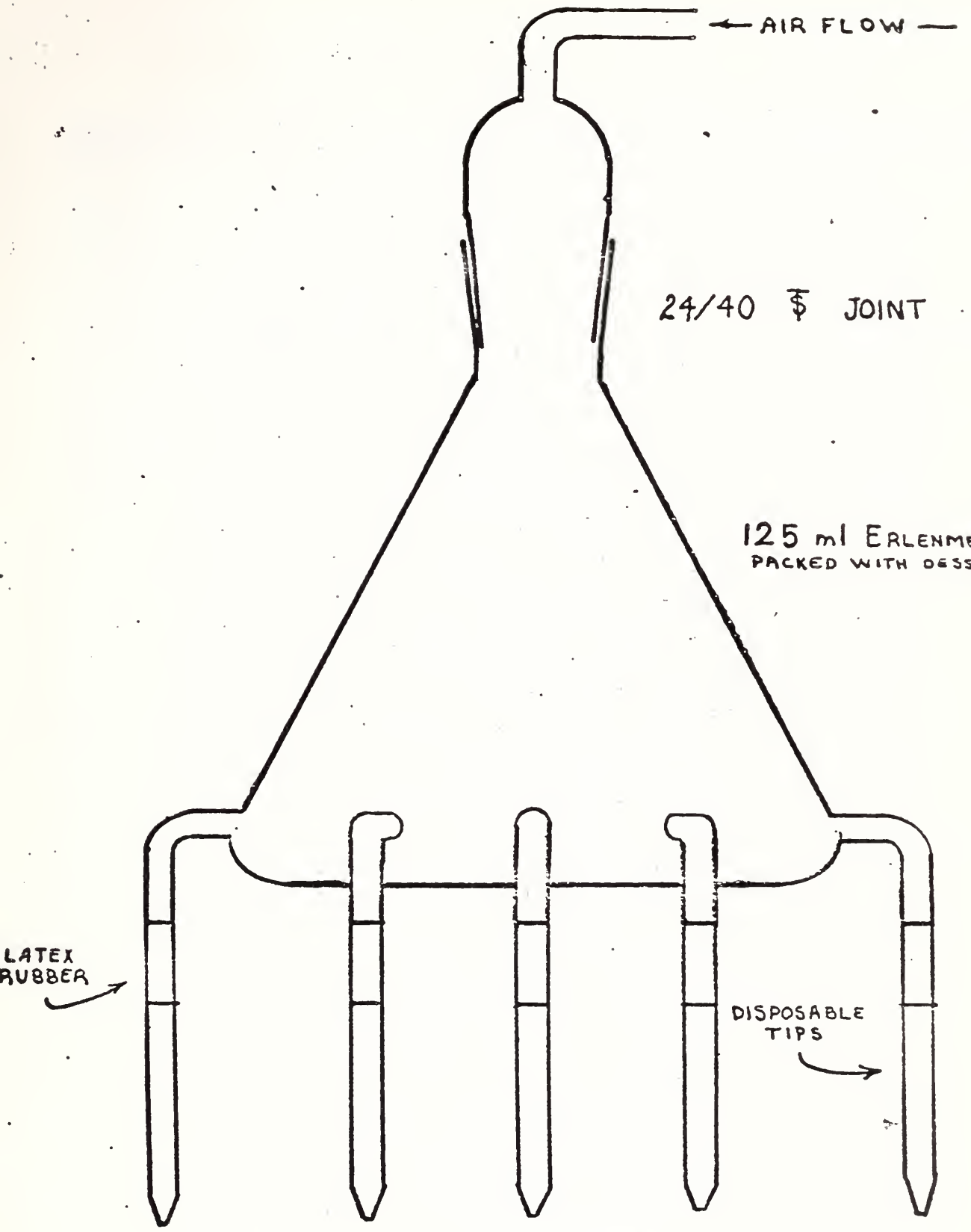
The following volumes of extracts are measured into a graduate cylinder and quantitatively transferred with washings to the column assembly.

Table I

Sample Type	Volume	g Sample Represented
Air	10 ml	
Biologicals > 20 g	15 ml	5
Biologicals < 20 g	entire extract	entire sample weight
Fats	15 ml	5
Soil and Sediment	5 ml	20
Vegetation	5 ml	5
Water	10 ml	1000

The eluant is collected in a 250 ml 24/40 S Erlenmeyer flask. The sample extract is drained to the top of the upper sodium sulfate layer, and 150 ml of a 5% v/v methylene chloride/hexane solution added. The eluant is collected until the solution level reaches the upper sodium sulfate layer. The flask containing this first fraction is replaced with a second flask and the column is eluted to dryness with 100 ml of methylene chloride. Mirex, heptachlor, p,p'DDT, and its metabolites are located in the first fraction, while dieldrin, endrin, and organophosphorous compounds are contained in the second fraction. Heptachlor epoxide appears in either fraction, depending on the particular batch of Florisil.

One ml of a 0.01% v/v Nujol solution and a few glass beads are added, and a three-ball Snyder column is attached to the flask. The eluant is concentrated to approximately 5 ml on an explosion-proof hot plate. To the second fraction 50 ml of hexane are added through the Snyder column and again concentrated to approximately 5 ml (this will remove all traces of methylene chloride). The eluant is quantitatively transferred with washings to a graduated centrifuge tube, and the final volume adjusted using a water bath and a stream of air (Fig 2) to the following volumes.



AIR CONCENTRATOR
FIGURE II

Table 2

Sample Type		Final Volume	g Sample Represented
Air		10 ml	
Birds		15 ml	5
Other Biologicals	20 g	5 ml	5
Other Biologicals	20 g	5 ml	Sample weight
Soil and Sediment		5 ml	20
Vegetation		5 ml	5
Water		10 ml	1000